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| (21) International Application Number: PCT/US93/10953 (22) International Filing Date: 12 November 1993 (12.11.93) (30) Priority data: 07/978,891 13 November 1992 (13.11.92) US (71) Applicant: IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121-1104 (US). (72) Inventors: ANDERSON, Darrell, R. ; 1851 Navato Place, Escondido, CA 92029 (US). RASTETTER, William, H. ; 16067 Puerta Del Sol, Rancho Santa Fe, CA 92067 (US). HANNA, Nabil ; 3255 Fortuna Ranch Road, Olivenhain, CA 92024 (US). LEONARD, John, E. ; 1960 Avenida Joaquin, Encinitas, CA 92024 (US). NEWMAN, Roland, A. ; 43111 Robbins Street, San Diego, CA 92122 (US). REFF, Mitchell, E. ; 4166 Combe Way, San Diego, CA 92122 (US). | (74) Agents: WEISS, Steven, M. et al.; Lyon & Lyon, 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US). (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i> | |
| (54) Title: THERAPEUTIC APPLICATION OF CHIMERIC AND RADIOLABELED ANTIBODIES TO HUMAN B LYMPHOCYTE RESTRICTED DIFFERENTIATION ANTIGEN FOR TREATMENT OF B CELL LYMPHOMA (57) Abstract <p>Disclosed herein are therapeutic treatment protocols designed for the treatment of B cell lymphoma. These protocols are based upon therapeutic strategies which include the use of administration of immunologically active mouse/human chimeric anti-CD20 antibodies, radiolabeled anti-CD20 antibodies, and cooperative strategies comprising the use of chimeric anti-CD20 antibodies and radiolabeled anti-CD20 antibodies.</p> | | |

C. Determination of Immunological Activity of Chimeric Anti-CD20 Antibodies

i. Human C1q Analysis

Chimeric anti-CD20 antibodies produced by both CHO and SP2/0
5 cell lines were evaluated for human C1q binding in a flow cytometry assay using
fluorescein labeled C1q (C1q was obtained from Quidel, Mira Mesa, CA, Prod.
No. A400 and FITC label from Sigma, St. Louis MO, Prod. No. F-7250; FITC.
Labeling of C1q was accomplished in accordance with the protocol described in
Selected Methods In Cellular Immunology, Michell & Shiigi, Ed. (W.H. Freeman
10 & Co., San Francisco, CA, 1980, p. 292). Analytical results were derived using a
Becton Dickinson FACScan™ flow cytometer (fluorescein measured over a range
of 515-545 nm). Equivalent amounts of chimeric anti-CD20 antibody, human
IgG1,K myeloma protein (Binding Site, San Diego, Ca, Prod. No. BP078), and
2B8 were incubated with an equivalent number of CD20-positive SB cells,
15 followed by a wash step with FACS buffer (.2% BSA in PBS, pH 7.4, .02% sodium
azide) to remove unattached antibody, followed by incubation with FITC labeled
C1q. Following a 30-60 min. incubation, cells were again washed. The three
conditions, including FITC-labeled C1q as a control, were analyzed on the
FACScan™ following manufacturing instructions. Results are presented in
20 Figure 6.

As the results of Figure 6 evidence, a significant increase in fluorescence was
observed only for the chimeric anti-CD20 antibody condition; ie only SB cells
with adherent chimeric anti-CD20 antibody were C1q positive, while the other
25 conditions produced the same pattern as the control.

ii. Complement Dependent Cell Lyses

Chimeric anti-CD20 antibodies were analyzed for their ability to
lyse lymphoma cell lines in the presence of human serum (complement source).
30 CD20 positive SB cells were labeled with ⁵¹Cr by admixing 100μ Ci of ⁵¹Cr with